DIGESTION OF FAT IN AMOEBA PROTEUS

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Greenwood (1886) maintains that if *Amoeba* comes in contact with milk, the fat globules in it are ingested but that they are not digested. She concludes that *Amoeba* does not digest fat. Nirenstein (1905) says there is no evidence which shows that fat is digested by any of the unicellular organisms.

Dawson and Belkin (1928–'29) injected various oils into *Amoeba proteus* and *Amoeba dubia* and then measured the diameter of the globules daily. They found that globules consisting of codliver, olive, cottonseed, linseed, sperm, peanut, oxfoot or oleic oil decreased in size in *Amoeba proteus* and globules of all these, except oxfoot and oleic oils, in *Amoeba dubia,* but that globules of paraffin oil did not decrease in either. They conclude that all these oils except paraffin are digested in *Amoeba proteus* and all except oxfoot, oleic and paraffin in *Amoeba dubia.* It is obvious, however, that the decrease observed in the size of the globules may have been due to oxidation rather than to digestion. Their results consequently do not prove that fat is digested in *Amoeba.*

Mast and Hahnert (1935) studied the digestion of *Chilomonas paramecium* in the food vacuoles in *Amoeba proteus.* They conclude that the fat globules pass from the chilomonads into the surrounding fluid in the vacuole and then out into the cytoplasm but that they probably do not undergo any chemical or physical changes during this process, i.e. that they probably are not digested. They maintain that after the globules of fat pass out of the food vacuoles, they become uniformly distributed through the cytoplasm and finally disappear, presumably owing to oxidation. They consequently obtained no evidence indicating that fat is digested by *Amoeba.*

The following observations were made on the fat in *Colpidium striatum* ingested by *Amoeba proteus.* The colpidia used were cultured in a solution which contained 15 grams tryptone and 2 grams KH_2PO_4 per 1,000 cc. water and an unidentified mold but no bacteria. The mold contained much fatty acid. The colpidia fed on the mold and contained an extraordinarily large amount of neutral fat. The amoebae used were taken from an old declining culture which still contained numerous

amoebae. These amoebae contained practically no fat and no food vacuoles except a few in which *Monas punctum* was found. This organism is not digested by *Amoeba proteus,* although it is often ingested in large numbers (Mast and Hahnert, 1935). Two methods were used.

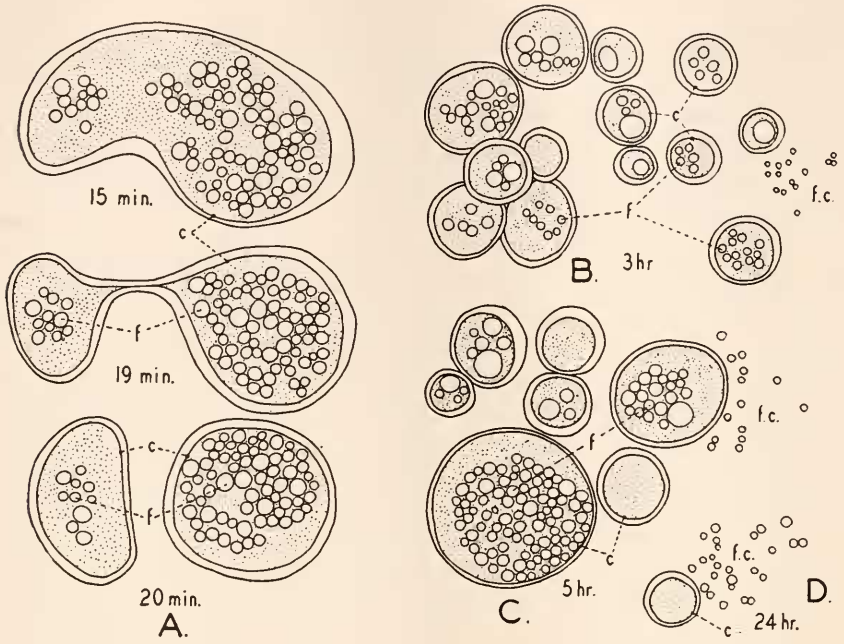

FIG. 1. Camera outlines of food vacuoles in *Amoeba proteus* containing *Colpidium striatum.* *c*, colpidia or fragments of colpidia in food vacuoles; *f*, globules of fat in the colpidia; *fc*, globules of fat in the cytoplasm; 15 min., 19 min., etc., time after ingestion.

A, division of the food vacuole and the colpidium in it; *B*, the food vacuoles and the fat in the cytoplasm of an amoeba three hours after it had ingested a colpidium (Note that the original food vacuole had divided into 13, each containing a fragment of the colpidium, that there were no globules of fat in the solution in the vacuoles around these fragments, that the fat in the cytoplasm of the amoeba was in the form of minute droplets, and that these droplets were much smaller than the globules of fat in the food vacuoles); *C*, the food vacuoles and the globules of fat in an amoeba five hours after it had ingested a colpidium (Note that the globules of fat in the cytoplasm of the amoeba had become larger but that they were still much smaller than those in the food vacuoles and that there was no decrease in size in those but that some vacuoles had none); *D*, the food vacuoles and the fat in the cytoplasm of an amoeba 24 hours after it had ingested a colpidium (Note that the globules of fat in the cytoplasm were well filled with fat and that the globules had increased considerably in size. Some of the amoebae examined had no food vacuoles and some had several but there was no fat in any of them).

I

About 250 amoebae were taken from the old culture referred to above. Several of these, taken at random, were stained for fat with Sudan IV. The rest were transferred successively through several separate portions of Hahnert solution;[1] then numerous colpidia were added and left 10 minutes, i.e., until most of the amoebae had ingested one or more colpidia, after which they were again passed through several portions of Hahnert solution so as to remove all the colpidia. An hour later several of the amoebae which contained colpidia were stained for fat with Sudan IV and examined under low and high magnification. This was then repeated from time to time for 24 hours. This entire experiment was repeated three times. There was no essential difference in the results obtained but there was great individual variation in the time required for the processes observed. The following results were obtained in one of the experiments.

1. Six amoebae were taken at random before the colpidia were added, and treated with Sudan IV. No fat was found in two, a few very small globules in one and a considerable number of very minute droplets which probably were fat in each of the rest.

2. Nine were taken 2 hours after having been fed with colpidia and treated with Sudan IV. Preliminary observations showed that soon after a colpidium is ingested the food vacuole usually divides several times, resulting in a number of small vacuoles each of which contains a fragment of the colpidium. Two of the nine amoebae had no food vacuoles but they had a considerable number of minute droplets scattered through the cytoplasm, which probably were fat. Seven had respectively 6, 6, 8, 9, 11, 15 and 21 food vacuoles containing colpidia in various stages of division. A few of these had no fat, but the rest were well filled with large globules. No fat was found in the solution in the food vacuoles, around the fragments of colpidia.[2] Three of the amoebae had a few small fat globules in the cytoplasm and the rest had

[1] Hahnert solution is composed of KCl .004 gram, $CaCl_2$ .004 gram, $CaH_4(PO_4)_2 \cdot 2H_2O$ .002 gram, $Mg_3(PO_4)_2 \cdot 4H_2O$ .002 gram, $Ca_3(PO_4)_2$ .002 gram, and H_2O 1,000 cc.

[2] Mast and Hahnert (1935) maintain that the globules of fat in chilomonads in food vacuoles in amoebae pass out into the surrounding solution in the vacuoles. In observation on colpidia in amoebae which were under cover-glasses with but little space between them and the slides I repeatedly saw globules of fat come out of the colpidia especially when pressure (even if only slight) was applied to the cover-glass, but I never saw any indications of it in preparations in which the cover-glasses were supported so as to leave abundance of space under them. It is probable, therefore, that the movement of globules of fat out of the chilomonads observed by Mast and Hahnert was due to external pressure.

minute droplets scattered through the cytoplasm, like those which had no food vacuoles.

3. Nine were taken 3.5 hours after having been fed. Two of these had no food vacuoles and probably no fat in the cytoplasm. The rest had respectively 4, 9, 12, 13, 15, 16 and 18 food vacuoles. Two of these had very minute droplets probably fat. Each of the rest had a considerable number of small globules of fat scattered through the cytoplasm but these globules were much smaller than those in the fragments of colpidia in the food vacuoles.

4. Nine amoebae were taken 5.5 hours after having been fed. All of these had food vacuoles, the number varying from 3 to 17. One of the amoebae had minute droplets in the cytoplasm, probably fat. Eight had small fat globules scattered through the cytoplasm.

5. Six amoebae were taken 7 hours after having been fed. All had food vacuoles, a total of 50. Eighteen of them had fragments of colpidia which contained large globules of fat. The rest (32) had no fat. Four of the amoebae had numerous small fat globules scattered through the cytoplasm. One had a moderate number and one only a few. None had minute droplets. All the globules of fat in the cytoplasm were much smaller than those in the food vacuoles.

6. Eight amoebae were taken 24 hours after having been fed. Only one of the 8 had any food vacuoles and that one had only four and there was no fat in any of them. One had no fat in the cytoplasm. All the rest had numerous globules scattered through the cytoplasm (Fig. 1).

The results presented show that soon after a colpidium is ingested the food vacuole and its content divides and that this is repeated until there are several, each containing a portion of the colpidium. They show that the globules of fat in the fragments of colpidia do not pass out into the solution around them in the vacuoles but that they gradually decrease in number until all have disappeared and that as they decrease in number, globules of fat appear in the cytoplasm of the amoeba and increase in size and number but that they are continuously much smaller than those in the food vacuoles.

This proves that the fat does not leave the food vacuoles in the form of globules and that it is digested, passes out into the cytoplasm in solution and is there resynthesized.

II

About 200 amoebae were taken from the old culture used in the preceding experiments and put into Hahnert solution; then numerous colpidia were added. After 2, 4, 9 and 24 hours respectively, some of the

amoebae which had ingested colpidia were taken out and put into Hahnert solution and stained with Nile blue sulfate for fat and fatty acid. Some colpidia which had not been ingested, some amoebae from a young culture and some emulsified oleic acid were also stained with Nile blue sulfate.

The oleic acid became densely purplish blue. The fat in the colpidia and that in the cytoplasm of the amoebae became pink, indicating that it was neutral. There was no bluish tinge in any of it, i.e. there was no indication of fatty acid. The fat in the colpidia which had recently been ingested was just as pink as that in the colpidia which had not been ingested and that in the cytoplasm of the amoebae. That in the colpidia which had been in the food vacuoles 2 or more hours was lavender or purple. The shade varied considerably in different vacuoles but it was *clearly* bluish in all. (The color of the fat in these vacuoles was repeatedly compared with that of the fat in the cytoplasm of the amoebae and that in colpidia which had recently been ingested, and it could definitely be seen that there was a marked difference in color, the former being definitely bluish.) The fat in the oldest (smallest) food vacuoles was, however, not appreciably bluer than that in younger vacuoles. It was not distinctly blue in any, not blue like the blue obtained in emulsions of oleic acid.

These facts indicate that the fat in *Colpidium* is changed to fatty acid and glycerine, that these substances leave the vacuoles soon after they are formed, and that in the cytoplasm of the amoebae they unite and form neutral fat. There is no evidence indicating that fatty acid accumulates to any considerable extent in the vacuoles. Small amounts of fat in old vacuoles are no bluer than large amounts in younger vacuoles.

Summary

1. If a colpidium which contains numerous globules of fat is ingested by an amoeba which contains no fat, the food vacuole and its content soon divide several times forming a number of vacuoles, each containing a fragment of the colpidium.

2. The globules of fat in these fragments gradually disappear and as they disappear, small droplets of fat appear in the cytoplasm of the amoeba, which gradually increase in size and number until in 24 hours all the fat in the fragments has disappeared and the cytoplasm of the amoeba is well filled with globules of it.

3. Treatment with Nile blue sulfate shows that the fat in the intact colpidia and that in the cytoplasm of the amoebae is neutral, but that

the fragments of colpidia in the food vacuoles contain fatty acid as well as neutral fat.

4. The fact that the globules of fat in the fragments of colpidia in the food vacuoles are much smaller than those in the cytoplasm of the amoebae when first observed, shows that they do not pass out into the cytoplasm and it indicates that the fat is digested in the fragments and passes out in solution. The fact that fatty acid is formed in the fragments indicates that the fat in them is split into fatty acid and glycerine which pass out into the cytoplasm and unite there to form neutral fat.

LITERATURE CITED

Dawson, J. A., and M. Belkin, 1928. The digestion of oils by Amoeba dubia. *Proc. Soc. Exper. Biol. and Med.*, **25**: 790–793.

Dawson, J. A., and M. Belkin, 1929. The digestion of oils by Amoeba proteus. *Biol. Bull.*, **56**: 80–86.

Greenwood, M., 1886–'87. On the digestive process in some rhizopods. *Jour. Physiol.*, **7**: 253–273, **8**: 263–287.

Hahnert, W. F., 1932. Studies on the chemical needs of Amoeba proteus: Culture method. *Biol. Bull.*, **62**: 205–211.

Mast, S. O., and W. F. Hahnert, 1935. Feeding, digestion, and starvation in Amoeba proteus (Leidy). *Physiol. Zoöl.*, **8**: 255–272.

Nirenstein, E., 1905. Beiträge zur Ernährungsphysiologie der Protisten. *Zeitschr. für allgemeine Physiol.*, **5**: 435–510.